## **Technical Data Sheet**

# РЕ-Cy™7 Rat IgG2a, к Isotype Control

### **Product Information**

Material Number:	557855
Size:	100 tests
Vol. per Test:	5 μl
Clone:	R35-95
Immunogen:	Mouse Pooled Immunoglobulin
Isotype:	Rat (LOU) IgG2a, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

#### Description

The R35-95 hybridoma was generated by hybridization of Y3 myeloma cells with spleen cells from LOU rats immunized with mouse immunoglobulins. The R35-95 hybridoma produces rat IgG2a,  $\kappa$  immunoglobulin that has no measurable reactivity with mouse immunoglobulins. The R35-95 immunoglobulin was selected as an isotype control following screening for low background binding on a variety of mouse and human tissues.

#### **Preparation and Storage**

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

#### **Application Notes**

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Flow cytometry	Routinely Tested	
Isotype control	Routinely Tested	

#### **Recommended Assay Procedure:**

An isotype control should be used at the same concentration as the antibody of interest (e.g.,  $\leq 1 \mu g/million$  cells for flow cytometry).

#### **Product Notices**

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^{6}$  cells in a 100-µl experimental sample (a test).
- 2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
- 6. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
- 7. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
- 8. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD<sup>™</sup> Stabilizing Fixative (Cat. No. 338036).
- 9. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
- 10. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- 11. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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References Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods*. 1995; 188(1):117-128. (Methodology)